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AQUAPORIN

DTU Technical University of Denmark

Introduction

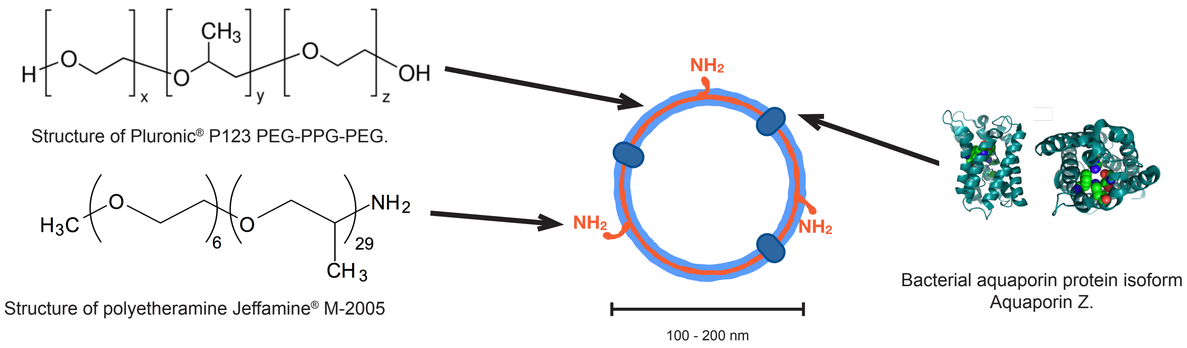

Figure 1. *Structure of polymeric vesicle functionally reconstituting AqpZ proteins, which protects the protein from degradation. The vesicle is made of amphiphilic ABA copolymer and equipped with NH_2 groups which will allow the covalent bonding into the final polyamide matrix of the membrane active layer.*

- Given the rising problem of water scarcity the significance of developing highly efficient membranes for water purification and reuse becomes increasingly important.
- Biomimetic membranes represent a highly efficient alternative to traditional membranes, due to improved water flux, without compromising on selectivity (Tang et al., 2013). These membranes mimic functional features of biological membranes by reconstitution of selected channel proteins such as aquaporin (Kumar et al., 2007).
- Aquaporin channels, like all proteins are very sensitive to their environment, here we propose a way to ensure protein functionality by incorporating Aquaporin Z (AqpZ) into polymer-based self-assembled nanostructures – vesicles / proteopolymersomes.
- The vesicles proposed here are Pluronic® based, formed by self-assembly of the amphiphilic block copolymer Pluronic® and Jeffamine® a hydrophobic amino modified polypropylene oxide mixture.
- Vesicle size and water permeability was quantified, subsequently they were embedded into forward osmosis (FO) polyamide thin film composite (TFC) membranes, which performance was characterized in terms of water flux (J_W) and reverse solute flux (J_S).
- **The aim of the study is to develop an formulation for polymeric nanostructures functionally reconstituting AqpZ proteins, which can be covalently incorporated into biomimetic membranes.**

Materials & Methods

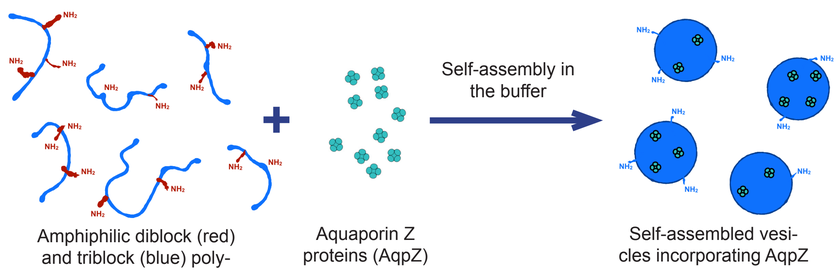

Figure 2. *Polymeric vesicles are prepared with use of Pluronic® P123 PEG-PPG-PEG, polyetheramine Jeffamine® M-2005, phosphate-buffer saline and AqpZ.*

The **dimensions of the vesicles (hydrodynamic diameter)** are determined by **dynamic light scattering** using ZetaSizer Nano ZS from Malvern. The **water flux through vesicle membrane** is tested using a **Bio-Logic SFM 300 stopped-flow (SF) device**, using a monochromator at 517 nm and a cut-off filter at 530 nm. For each individual SF test, 0.13 ml polymersomes or AqpZ embedding polymersomes samples, were quickly mixed with 0.13 ml NaCl 0.5 M, which caused water efflux from vesicles resulting in vesicle shrinkage.

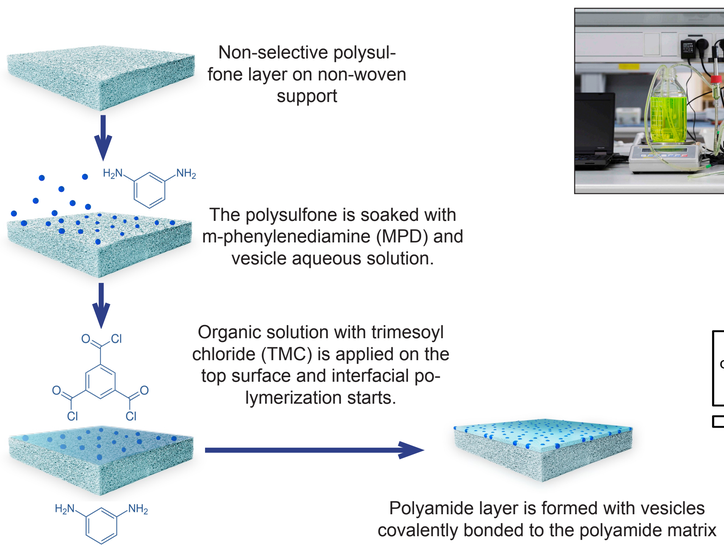

Figure 3. *Preparation of the active layer of the membrane.*

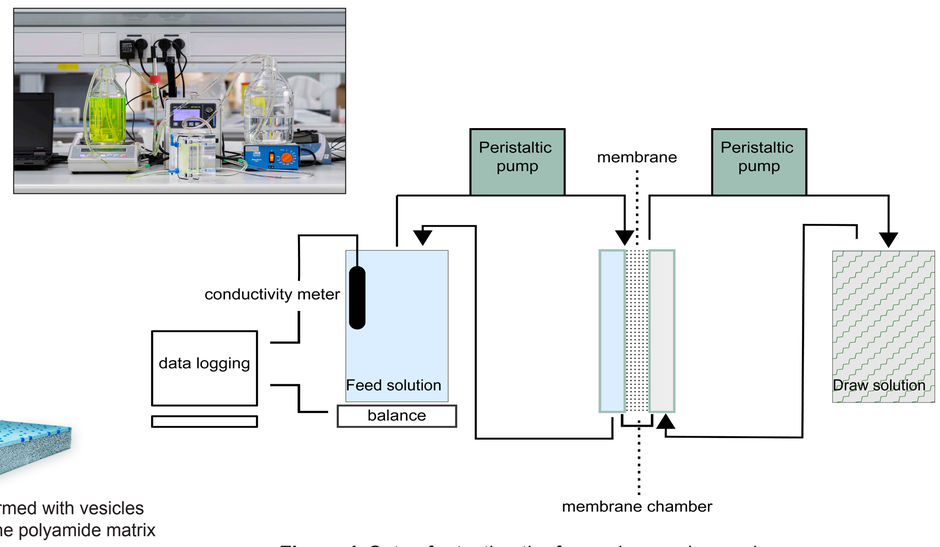

Figure 4. *Setup for testing the forward osmosis membrane performance, draw solution used is 1 M NaCl.*

Results & Discussion

Table 1. Characteristics of Pluronic® vesicles.

	Hydrodynamic diameter [nm] - % Intensity		Zeta potential [mV]	pH	Osmotic coefficient k_i [s^{-1}]
	Population 1.	Population 2.			
Pluronic® vesicles reconstituting Aquaporin Z	158 ± 63 - 93 %	34 ± 8 - 7%	+ 3	9.91	1700
Pluronic® vesicles without Aquaporin Z	147 ± 49 - 95%	33 ± 6 - 5%	+ 3	9.92	200

The **only significant change in vesicles characteristics when AqpZ proteins are incorporated is the osmotic coefficient**, which is directly related to the water flux through the membrane of the vesicle (Grzelakowski et al., 2015).

$$Y = at + b + \sum_{i=1}^{N} c_i \, e^{-k_i t}$$

Equation 1. *Exponential equation used for calculation of the osmotic coefficient, where:*
t - time [s]
k_i - osmotic coefficient [s^{-1}]
c_i - relative amplitude
a; b - fitting coefficients
Y - light scattering

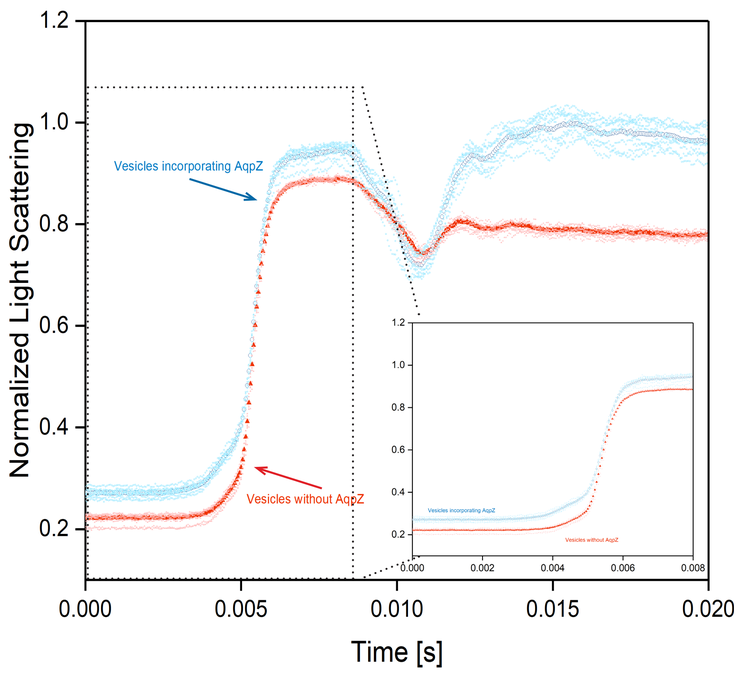

Figure 5. *Stopped-flow light scattering results for the vesicles incorporating Aquaporin Z and blank ones. The analysis of exponential growth is made on the first population of the structures showing the most rapid shrinkage.*

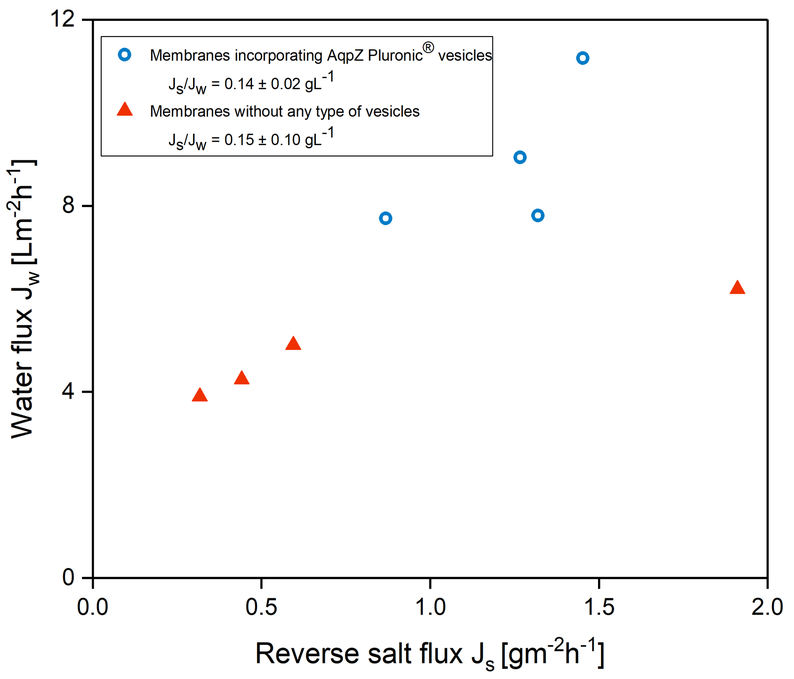

Figure 6. *The screening FO experiment with membranes incorporating Pluronic® vesicles incorporating AqpZ.*

- The preparation of vesicles by self-assembly is advantageous over film rehydration method, often used for polymersomes preparation, as it requires less equipment and is faster.
- The **method for preparation of vesicles that we propose here is simple and does not require filtration step**, that is very often necessary when dealing with self-assembly of polymeric vesicles.
- Vesicles proposed here, however, also have disatvantages such as limited long-term stability in diluted aqueous solutions, as the vesicles will tend to dissasemble to other nanostructures, when concentrations of polymers are changed. This is due to the fact that the form of vesicles will no longer be the most energy-favourable.

Conclusions

- **AqpZ transmembrane water channel proteins can be succesfully functionally reconstituted into PEG-PPG-PEG copolymer membrane of the vesicle.** Functional reconstitution has been confirmed by stopped-flow, while DLS and Zeta-potential analysis confirmed that there is no significant effect on the size and electrokinetic properties of vesicles when AqpZ are incorporated in comparison to the AqpZ-free Pluronic® vesicles.
- Based on the screening experiment, it is shown that the incorporation of **AqpZ-reconsituting Pluronic® vesicles into the active polyamide layer of the membrane increase the flux over the FO membrane, without comprising on overall membrane performance (J_s/J_w).**

Outlook

- To understand in depth the incorporation of Pluronic® vesicles into the polyamide layer, further investigation on membranes has to be conducted - including testing in the reverse osmosis and vesicle quantification on the membrane.
- Furthermore, the proposed technology may be applied to the new generation of both reverse and forward osmosis biomimetic membranes, including the hollow fibre configuration.

References

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Acknowledgments

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Innovation Fund Denmark